AN EVALUATION ON THE EFFECT OF 2% CHLORHEXIDINE DIGLUCONATE ON MICROTENSILE BOND STRENGTH OF ULTRAMILD SELF ETCH ADHESIVES

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CERTIFICATE

This is to certify that this dissertation titled "**An Evaluation On The Effect Of 2% Chlorhexidine Digluconate On Microtensile Bond Strength Of Ultramild Self Etch Adhesives"** is a bonafide record of work done by **Dr. Divya. R** under my guidance and to my satisfaction during her postgraduate study period between 2010 – 2013. This dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in partial fulfillment for the award of the degree of Master of Dental Surgery in Conservative Dentistry and Endodontics, Branch IV. It has not been submitted (partial or full) for the award of any other degree or diploma.

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CONTENTS

ABSTRACT AND KEYWORDS

BACKGROUND:

Adhesive restorations are in much demand in the present day. The success of these restorations depends on the bond between the resin and the dentin provided by the adhesive used. Self-etch adhesives, which are becoming increasingly more popular, have been shown to suffer from hydrolytic bond degradation. Matrix metalloproteinases (MMPs) that are present in the dentin and saliva may be partially responsible for hybrid layer degradation. Since chlorhexidine inhibits MMPs, we hypothesized that chlorhexidine would decelerate the loss of resin-dentin bonds.

AIM:

The current study evaluated the effectiveness of 2% Chlorhexidine digluconate on the immediate bond strength of two ultra mild self etch adhesives, Clearfil S3 Bond and G Bond.

MATERIAL AND METHODS:

Four groups of tooth samples were evaluated, **Group I**: Clearfil S3 Bond, **Group II**: Clearfil S3 Bond with 2% Chlorhexidine digluconate Solution, **Group III**: G-Bond, **Group IV**: G-Bond with 2% Chlorhexidine digluconate Solution. After the application of the adhesives on the tooth samples according to their respective groups composite build up was performed. They were then sectioned to obtain resin dentin sticks of 1.0 ± 0.1 mm^2 which were mounted on a jig and tested for microtensile bond strength. The fractured specimens were then viewed under scanning electron microscope and the failure modes were evaluated. Bond strength values were evaluated using students 't' test.

RESULTS:

The results of the study showed no statistically significant differences in the samples bonded with G bond. Whereas in case of Clearfil S3 bond, the samples without Chlorhexidine

showed better bond strength than the samples bonded with Chlorhexidine. Adhesive failures occurred in the samples bonded with G Bond and cohesive failures were observed in case of Clearfil S3 Bond.

CONCLUSION:

Within the limitations of this study, it can be concluded that the use of 2% Chlorhexidine digluconate does not have much effect on the immediate bond strength of the specimens bonded with ultra mild self etch adhesives. However further in vivo studies should be carried out, to assess the long term effects of using 2 % Chlorhexidine digluconate on preservation of resin dentin bonds.

Keywords: Microtensile Bond strength, 4 MET, 10 MDP, Matrix metalloproteinase, 2% Chlorhexidine, scanning electron microscope.

INTRODUCTION

There was an era during the evolution of mankind when cavities in teeth were filled with stone chips, ivory, human teeth, cork, metal foils (lead, tin) or gold bands and amalgam [2]. Today at this juncture when the science of dentistry has scaled heights never before achieved, we have a new restorative material that has established itself as the undefeated champion in its league. Resin restorations are today's most preferred restorative material owing to a vast number of its desirable properties [6]. Even so nothing is without its imperfections and so resin restorations are no exception to the rule for they also have their very own Achilles' heel.

The longevity of resin restorations is currently an area of interest in adhesive dentistry. Throughout the last two decades, chemical and technical advances have contributed to increased resin-dentin bond strength. However, premature loss of bond strength is one of the problems that still affects adhesive restorations [32] and markedly reduces their durability [9]. It has been widely stated that resin-dentin bonds obtained with contemporary adhesive systems can deteriorate over time [38] and durability of the bond between dentin and resinous adhesives may not be as durable as was previously assumed [22, 15].

Today's focus in adhesive technology is directed towards simplified bonding procedures. This alternative approach is based on the use of non-rinse acidic monomers that simultaneously condition and prime dentin, the so called *'self-etch' adhesives*. Regarding user-friendliness and technique

sensitivity, this approach seems clinically most promising. This approach eliminates the rinsing phase, which not only lessens the clinical application time, but also significantly reduces the technique-sensitivity or the risk of making errors during application. There are basically three types of 'self-etch' adhesives viz 'Strong', 'Mild', 'Ultra mild' [53]. 'Strong' self-etch adhesives have a very low pH (< 1) and exhibit a bonding mechanism and interfacial ultra-morphology in dentin resembling that produced by etch and rinse adhesives. 'Mild' self-etch adhesives (pH of around 2) dissolve the dentin surface only partially, so that a substantial number of hydroxyapatite crystals remain within the hybrid layer. 'Ultra mild' self etch adhesives with a pH of >2.5 have a nano interaction depth. Compositions of the self etch adhesives plays an important role in the bond stability. Specific carboxyl or phosphate groups of functional monomers can chemically interact with the residual hydroxyapatite in the substrate. This two-fold bonding mechanism (*i.e.*, micro-mechanical and chemical bonding) was believed to be advantageous in terms of restoration durability.

Research has shown that one-step self-etch adhesives are more commonly associated with lower bonding effectiveness to both enamel/dentin than are their multi-step counterparts [3]. This could be attributed to the varied functional monomers in each of these adhesives that chemically interact with hydroxyapatite that remains within submicron hybrid layers produced by mild self-etch adhesives. The specific molecular nature of the functional monomer

and the subsequent dissolution rate of its calcium salt have been shown to determine actual chemical bonding efficacy and stability.

Among functional monomers used in commercially available adhesives, 4-methacryloxyethyl trimellitate anhydrate (4-META) or 4-methacryloxyethyl trimellitic acid (4-MET) and 10-methacryloyloxydecyl dihydrogen phosphate (10 MDP) are most commonly used acidic functional monomers. 4-MET, phenyl P and 10 MDP are the active ingredients of many currently available self-etch adhesives [59, 24]. In contrast to etch and rinse adhesives that involve phosphoric-acid etching, self-etch adhesives containing 4-MET, Phenyl P only partially demineralize the dentin, leaving hydroxyapatite partially attached to collagen within a submicron hybrid layer [59,24]. It has been suggested that this residual hydroxyapatite may serve as receptor for chemical interaction with the functional monomer, subsequently contributing to the eventual adhesive performance in addition to micro-mechanical hybridization. The functional monomer 10-methacryloyloxydecyl dihydrogen phosphate or 10-MDP, will chemically bond to calcium of hydroxyapatite of dentin forming a stable calcium-phosphate and calcium carboxylate salts, respectively, along with only a limited surface-decalcification effect [59].

The loss of bond strength has mainly been attributed to degradation of the hybrid layer at the dentin-adhesive interface. It has been speculated that resin monomer diffusion within the acid-etched dentin and a subsequent resin

elution from hydrolytically unstable polymeric hydrogels within the hybrid layers results in exposed collagen fibrils. These unprotected collagen fibrils are vulnerable to degradation by endogenous matrix metalloproteinases (MMPs) [57]. Recent findings indicate that MMP -2, -3, -8, -9 and -20 are present within the human dentin matrix which are very capable of degrading the organic matrix of demineralized dentin [49]. This ultimately leads to a reduction in the bond strengths [21, 8]. Human dentin matrices exhibit variable collagenolytic and gelatinolytic activities when mixed with dentin/enamel bonding agents with different pH's [35]. Thus, the simple application of adhesive systems on acid-etched dentin substrate can activate dentinal MMPs to initiate autolytic phenomena that will eventually affect the hybrid layer.

Dentin collagenolytic and gelatinolytic activities can be suppressed by protease inhibitors [38], indicating that MMP inhibition could be beneficial in the preservation of hybrid layers. This was demonstrated in a recent study, in which the application of chlorhexidine, known to have a broad-spectrum MMP-inhibitory effect [19], significantly improved the integrity of the hybrid layer in a six-month clinical trial [22]. Chlorhexidine is known to be an inhibitor of MMP activity *in vitro* [38, 19]. However, in an *in vivo* study, [22] an experimental group included the application of a 2% solution of chlorhexidine digluconate to primary dentin after acid etching. Teeth from this group showed less degradation of the hybrid layers than the control group, although the study evaluated only a small number of teeth. CHX prevents or minimizes the auto-

degradation of exposed collagen fibrils within incompletely-formed hybrid layers, thereby contributing to the long-term stability of the hybrid layer and bond strength [22]. Additionally, CHX may also be a useful complementary method to other techniques of proven efficacy for rehydrating dried mineralized dentin and therefore, preserving the humidity necessary for keeping the collagen network expanded.[43] However, any positive benefits would be negated if CHX interferes with a hydrophilic resin's ability to wet and micromechanically bond to dentin.

Hence the effect of 2% Chlorhexidine Digluconate on the 2 functional monomers i.e. 10 MDP and 4 MET with Phenyl P have to be evaluated for the above said rationale.

AIM AND OBJECTIVE

The purpose of the current study was to evaluate the effectiveness of 2% Chlorhexidine Digluconate on immediate microtensile bond strength (μTBS) of two ultra mild self etch adhesives.

REVIEW OF LITERATURE

Buonocore (1954)[7] attempted to obtain bonding between filling materials and tooth structure. These included the development of new resin materials which have adhesive properties, the use of coatings as adhesive interface materials between filling and tooth and the alteration of the tooth surface by chemical treatment to produce a new surface to which present materials might adhere. It was concluded that the increased adhesion can be obtained by acid etching enamel surfaces using phosphoric acids. Formations of precipitation of calcium oxalate, organic tungstate complexes on the surfaces of the tooth to which acrylic can adhere were deemed useful for adhesion.

Van meerbeek et al (1993)[52] evaluated the cross-sectioned resin-dentin specimens that were bonded with a 5-NMSA (N-methacryloyl 5-aminosalicylic acid) and 10 MDP (10-methacryloyloxydecyl dihydrogen phosphate) based adhesive system after dentin pretreatment with an aqueous acid solution (10% citric acid and 20% calcium chloride). Ultrastructural evaluation of the interdiffusion zone was assessed using Scanning and Transmission electron microscopic analysis. The results indicated that the diffusion of resin monomer into the demineralized dentin decreases with depth. It was concluded that the acidic pretreatment of the dentin caused denaturation of the superficial collagen fibrils.

Pashley et al (1995)[37] reviewed on the testing methodologies employed for In vitro testing of the strength of the bonding agents. He described the importance of variables involved like etching, priming and bonding, storage

variables, testing variables, the adhesion substrate and dentin. Recent advances in the development of newer bonding systems and their testing modalities were described. The author described that higher dentin bond strengths develop non uniform stress distributions in dentin during in vitro testing, causing cohesive failures in the substrate rather than in the bonded interface. It was concluded that conventional bond testing methods can no longer be used to detect further improvements in product development or bonding procedures. Hence newer testing methods like microtensile bond strength testing provide a more accurate measurement of the bond strength.

Burrow et al (1996)[8] determined the durability of tensile bond strength over 3 yrs to bovine dentin using the dual cured bonding resin (Clearfil Photobond), without the placement of a priming agent or with 5 NMSA priming agent. The bonded interface was then observed using a Field emission scanning electron microscope. The results indicated that greater bond strength was obtained for the primed group compared to the unprimed group. It was concluded that priming may only be useful to achieve strong bonding in the short term.

Tjaderhane et al (1998)[49] investigated the presence and the potential functional activity of MMPs in human soft dentinal caries lesions, in addition whether human MMPs could participate in the degradation of dentin organic matrix after demineralization. Western blot analysis using MMP-specific antibodies for dental caries, enzymography and functional activity assays were

performed. The results demonstrated that MMP-9, MMP-2, and MMP-8 were activated at low pH followed by neutralization. These low pH levels present in bone resorption lacunae and dental caries lesions were sufficient to activate the latent MMPs.

Gendron et al (1999)[19] evaluated the inhibitory effect of therapeutically attainable concentrations of CHX on the activities of MMP -2, -8, and -9. The effects of chlorhexidine on MMP -2, -8, -9 were evaluated using cellular inhibition assays. The results indicated that CHX inhibited the activities of both gelatinases but MMP-2 appeared to be more sensitive than MMP-9. CHX dose-dependently inhibited collagenolytic activity of the released MMP-8. It was concluded that the inhibition of MMPs by CHX demonstrates new beneficial antiproteolytic properties and has potential clinically advantageous anti MMP properties.

Tay and Pashley (2001) [46] evaluated the aggressiveness of three self-etching adhesive systems in penetrating dentin smear layers of different thickness, with the use of Transmission Electron Microscopy (TEM). The results indicated that three self-etching adhesive systems exhibited different degrees of aggressiveness in their ability to demineralize subsurface intact dentin. It was concluded that self-etching systems may be classified into mild, moderate and aggressive based on their ability to solubilize dentin smear layers and the demineralization of the subsurface dentin.

Tay and pashley (2002)[47] reviewed on the current trends in the development of the newer dental adhesives. The older dental adhesives had major drawbacks like unpredictable bond strength and were highly technique sensitive. These shortcomings were overcome by developing newer adhesives with simplified bonding steps making them more user friendly. Although dentin adhesives have improved tremendously over the past decade, postoperative sensitivity, incomplete marginal seal, premature bond degradation, biocompatibility, and compromised bonding to abnormal substrates are still considered potential problems associated with their use. With the advances in biomimetics, future dentin adhesive monomers may contain domains derived from underwater bio adhesives. It may also contain fluorescent biosensors that can detect pH changes around leaking restorations. The future dentin adhesives may assume a more instrumental role in therapeutics apart from just caries prevention.

Castro et al (2003)[14] evaluated the effects of 2% chlorhexidine on the microtensile bond strength of composite resin to dentin treated with three dentin bonding systems (Prime & Bond NT, Single Bond and Clearfil SE Bond). It was concluded that 2% chlorhexidine solution, applied before or after acid etching of the dentin, did not interfere with the microtensile bond strength of composite resin to the dentin treated with bonding systems.

Ferrari et al (2003)[18] examined the ultrastructure of the resin dentin interface and the extent of tracer penetration created in deep, vital acid-etched

dentin under different degrees of hydration of the demineralized collagen matrices using transmission electron microscopy. This study hypothesized that there is no difference between moist bonding performed in vitro or in vivo, and that excessive drying or wetting of vital acid-etched dentin produces inferior results. The results of the study indicated that the patterns of silver deposition created in vitro or in vivo were similar within the adhesive and hybrid layers. No hybrid layer was observed in vivo after excessive drying. Excessive wetting in vivo resulted in more extensive nanoleakage and water tree formation along resin-dentin interface. It was concluded that the type of bonding procedure performed may eventually result in an increase in the rate of degradation of resin-dentin bonds created in vital dentin.

Hashimoto et al (2003)[21] determined the biodegradation of resin–dentin bonds after exposure to water for one year, using the combined methodologies of microtensile bond strength testing, scanning electron microscope observations of the fractured surfaces and interfacial observations by transmission electron microscope. The results indicated that two degradation patterns were observed within hybrid layers after storage in water for 1 year, the disorganization of the collagen fibrils and loss of resin from interfibrillar spaces within the hybrid layer. Such degradation thereby resulted in weakening of resin–dentin bond leading to bond strength reduction.

Osorio et al (2003)[36] evaluated the microleakage of Class V resin composite restorations made with three different adhesive systems: two

containing acidic primers and a conventional three step adhesive system. The prepared specimens were then immersed in a solution of 2% basic fuchsin dye. Longitudinal sections of the specimens were obtained and studied with a stereomicroscope for assessment of the microleakage according to the degree of dye penetration. The results of the current study states that the use of self etching primers in Class V composite restorations can achieve a marginal integrity comparable (on enamel) or better (on dentin) to that attained by the conventional conditioning of the enamel with phosphoric acid. Combining conditioning and priming into a single treatment step results in an improvement in both time and cost effectiveness.

Wang and Spencer (2003)[57] determined the quality and molecular structure of adhesive and dentin interfaces formed with wet bonding as compared with adhesive-infiltrated demineralized dentin (AIDD) produced under optimum hybrid conditions. The null hypothesis tested was that adhesive resin applied under wet-bonding conditions enveloped the exposed collagen fibrils, forming a structurally integrated hybrid layer at the molecular level. From each extracted, unerupted human 3rd molar, one fraction was demineralized, dehydrated, and infiltrated with Single Bond (SB) adhesive under optimum conditions; the remaining adjacent fraction was treated with SB by wet bonding. Sections were then analyzed with Micro- Raman spectroscopy. The results indicated that under wet bonding, the adhesive dentin interface is a porous collagen web infiltrated primarily by the hydrolytically unstable HEMA.

Pashley et al (2004)[38] determined whether acid-etched dentin matrices can be degraded by dentin-derived proteolytic enzymes, in the absence of bacterial colonization over time. These dentin matrices were aged in artificial saliva containing proteolytic enzyme inhibitors and in non-aqueous mineral oil. The results indicated that the thickness of the remnant demineralized collagen matrix and the status of the collagen fibrils were different when acid-etched dentin was aged in the experimental and the two control storage media. It was concluded that hydrolytic degradation of denuded collagen fibrils occured in the absence of bacterial colonization. From the results of the study it was hypothesized that it would be advantageous to prevent the degradation of incompletely resin-infiltrated collagen fibrils by host-derived MMPs in dentin hybrid layers.

Yoshida et al (2004)[59] comparatively characterized the adhesive interaction of 3 functional monomers (10 MDP, Phenyl P and 4 MET) with synthetic hydroxyapatite to test the hypothesis that the bonding mechanism of mild self-etch adhesives involves chemical interaction of functional monomers with residual hydroxyapatite in addition to micro-mechanical hybridization. The adhesive interactions of 3 functional monomers were tested with synthetic hydroxyapatite, using x-ray photoelectron spectroscopy and atomic absorption spectrophotometry and further interaction with dentin was ultra-morphologically viewed using transmission electron microscopy. The results indicated that the bonding potential of 4 MET was lower when compared to that

of 10 MDP. The bonding potential of phenyl P was hydrolytically unstable. It was concluded that the specific functional monomers have additional chemical bonding efficacy which is expected to contribute to their adhesive potential to tooth tissue.

De Munck et al (2005)[16] determined the interaction to enamel/dentin of contemporary one- and two-step self etch adhesives and was compared to a control group, two and three step etch and rinse adhesive. The bonding effectiveness was measured by microtensile bond strength testing, Field-emission scanning electron microscopy and Transmission electron microscopy. The results of the study indicated that the one-step self-etch adhesive scored the lowest microtensile bond strength of all experimental and control adhesives tested. Ultramorphological characterization showed that interfacial morphology and the pH of the self etch primer/adhesive is strongly associated with the bond strength of the material.

Hebling et al (2005)[22] assessed the endogenous collagenolytic and gelatinolytic activities derived from the acid etched dentin in degradation of hybrid layer and demonstrated that such activities could be arrested via the use of chlorhexidine as an MMP inhibitor applied after phosphoric acid-etching but before adhesive application. The results indicated that the hybrid layers from the chlorhexidine-treated teeth exhibited normal structural integrity of the collagen network. Abnormal hybrid layers were seen in the control teeth, with progressive disintegration of the fibrillar network. It was concluded that self-

destruction of collagen matrices occurs rapidly in resin-infiltrated dentin *in vivo* and may be arrested with the use of chlorhexidine as an MMP inhibitor.

Inoue et al (2005)[24] determined the hydrolytic stability of 3 self etch adhesives that contained one of the 3 functional monomers (10 MDP, Phenyl P and 4 MET), through measurement of their micro-tensile bond strength (μTBS) to dentin. The characterization of the aged adhesive-dentin interface was evaluated by transmission electron microscope (TEM). The results indicated that the functional monomer 10-MDP interacted most intensively with hydroxyapatite and its calcium salt appeared most hydrolytically stable, as compared with 4-MET and phenyl-P. The findings of this study hence supported the concept that long-term durability of adhesive dentin bonds depend on the chemical bonding potential of the functional monomer.

Nishitani et al (2006)[35] compared the ability of several self-etching adhesives to increase the gelatinolytic and collagenolytic activities of mineralized dentin powder. Powdered dentin made from human teeth was mixed with all-in-one adhesives or a self-etching primer for varying times (20 sec or 5 min) and then the reaction was stopped by extracting the adhesives using acetone. The results of this study suggest that self-etching adhesives were sufficiently acidic to activate gelatinolytic and collagenolytic activities in mineralized dentin powder.

Tay et al (2006)[48] tested the hypothesis that latent collagenolytic activity is activated by mild self etch adhesives in instrumented intraradicular dentin.

Dried dentin powder aliquots were then treated with two clinically-relevant MMP inhibitors, 2% chlorhexidine for 10 minutes and 17% EDTA for 1 minute. The results indicated that instrumented, mineralized intra radicular dentin possessed low but detectable collagenolytic activity that was inhibited by chlorhexidine. It was concluded that mild self-etching adhesives activates latent MMPs without denaturing these enzymes and may adversely affect the longevity of bond.

Toledano et al (2006)[50] evaluated the differences in dentin bond strength when using three total etch (Single Bond, Prime & Bond NT and the experimental Prime & Bond XP) and two self-etching agents (Clearfil SE Bond and Etch&Prime 3.0) and that NaOCl immersion of specimens does not affect bond strength to dentin. The results indicated that Clearfil SE Bond and Single Bond attained higher MTBS than the other three adhesives. Etch & Prime resulted in the lowest MTBS. After NaOCl immersion, MTBS decreased in all groups. The highest MTBS values were obtained for Clearfil SE Bond and Prime&Bond XP. It was concluded that resin–dentin bonds were prone to chemical degradation and the tested all-in-one adhesive, provided the least durable bond strength. Resin dissolution occurs at the hybrid layer leaving collagen unprotected. The rate of resin dissolution is adhesive system specific.

Brackett et al (2007)[4] determined the rate of degradation and effect of 2% Chlorhexidine digluconate application on the immediate and 6 month in vivo bond strength. Class I cavities were prepared in caries free premolars

scheduled for orthodontic extraction and composite restorations were performed. Based on the results it was concluded that application of 2 % Chlorhexidine digluconate appeared to slow down the resin dentin bond degradation.

Carrilho et al (2007)[11] evaluated the effect of protease inhibition with the use of chlorhexidine on resin-dentin bond strength after 6 months of aging. The specimens were stored in artificial saliva with/without protease inhibitors. Microtensile bond strength and Scanning electron microscopic analysis were performed immediately after specimen preparation and 6 months later. The results of the study indicated that chlorhexidine, had significantly better preservation of bond strength after 6 months. It was concluded that chlorhexidine might be best for time related preservation of hybrid layer in turn leading to better bond strength.

Breschi et al (2008)[5] critically discussed the latest peer-reviewed reports related to formation, aging and stability of resin bonding which also focused on the micro and nano-phenomena related to adhesive interface degradation. The results indicated that most simplified one-step self etch adhesives were shown to be the least durable, while three-step etch-and-rinse and two-step self-etch adhesives demonstrated the highest performances. The author suggests that simplification of clinical application procedures might be detrimental to the bonding efficacy. Different aging phenomena occur at the dentin bonded interfaces which are considered pivotal in degrading the hybrid layer,

particularly in cases where simplified adhesives are used. The factors that reduce the longevity of the bonded interface are insufficient resin impregnation of dentin, high permeability of the bonded interface, sub-optimal polymerization, phase separation and activation of endogenous collagenolytic enzymes.

Soares et al (2008)[43] evaluated the effect of bond strength to bovine dentin on the application of chlorhexidine on the dentin substrate at different times. Superficial dentin of the bovine teeth was subjected to 0.12% and 2% chlorhexidine solutions for 15 seconds before, during and after 37% phosphoric acid etching. It was concluded that the use of chlorhexidine at concentrations of 0.12% and 2% before, after or associated with acid etching did not significantly affect the μTBS values to dentin.

Breschi et al (2009)[6] investigated the effects of long-term usage of 0.2% and 2% CHX on the mechanical durability of resin-bonded dentin treated with two simplified etch-and-rinse adhesives (Adper Scotchbond and XP-Bond). Transmission electron microscopic analysis was done to investigate the interfacial nanoleakage expression of the dentin bonded interface. The results indicated that the use of chlorhexidine as a primer on acid etched dentin even at a low concentration prevented degradation of the hybrid layer. Nanoleakage increased during aging in controls, but reduced silver deposits were found in CHX-treated specimens.

Carrilho et al (2009)[10] evaluated the changes in mechanical, biochemical and structural properties of demineralized dentin treated with and without chlorhexidine. Demineralizing of the dentin beams was done using either EDTA or Phosphoric acid incubated in artificial saliva. Based on the results of the current study it was concluded that lack of significant changes in the stiffness of the dentin and lower release of collagen peptides were seen in the specimens that were treated previously with chlorhexidine. This was due the inhibition of intrinsic MMP activity.

De Munck et al (2009)[17] hypothesized that adding MMP inhibitors to adhesive primers could prevent the endogenous enzymatic degradation, thereby improving the bond durability. A non-specific MMP inhibitor (chlorhexidine) and a MMP-2/9- specific inhibitor (SB-3CT) were admixed to the primers of etch & rinse and a self-etch adhesive. The results of the study indicated that the built-in MMP inhibitors appeared effective in reducing bond degradation only for etch & rinse adhesive, and not for the self-etch adhesive. The authors suggested that water sorption of adhesive interfaces most likely remains the principal mechanism of bond degradation for self etching adhesive systems.

Komori et al (2009)[25] evaluated the effect of a 2% chlorhexidine digluconate primer (CHX) on the durability of resin-dentin bonds in normal versus caries affected dentin, using three-step or two-step etch-and rinse adhesives. The results of the study indicated that the application of 2% CHX did not affect the immediate bond strength to the normal or caries affected dentin

with the use of etch-and-rinse adhesives. CHX treatment significantly lowered the loss of bond strength after six months for normal dentin but it did not alter the bond strength of caries affected dentin.

Lehmann et al (2009)[28] evaluated the possible changes in MMP-2 and MMP-9 expression in odontoblasts and pulp tissue after a self-etching adhesive treatment on dentin. In the cultured tooth slices the changes in expression of MMP's in the dentin after self-etching adhesive treatment was evaluated by immunochemistry and zymography. It was concluded that self-etching adhesive stimulates the secretion of MMPs from the dentin-pulp complex and more precisely, by odontoblasts suggesting that it participates in hybrid layer degradation.

Loguercio et al (2009)[29] evaluated the effects of 0.002, 0.02, 0.2, 2, and 4% concentrations of CHX and application times of 15 and 60 seconds on the durability of the dentin bonds. The bond strength of the resin dentin bond was examined at immediate and 6-month water storage and the silver nitrate uptake pattern of two-step etch-and-rinse adhesives. The results of the present investigation imply that a concentration of 0.002% of chlorhexidine applied for 15 seconds is sufficient to preserve the resin dentin bonds for 6 months.

Albaladejo et al (2010)[1] evaluated the hybrid layer, resin tags and adhesive lateral branches formations of five adhesive systems (two etch and rinse systems and three self etch) bonded to dentin. The author described the morphological characteristics and ascertained the probable relationship with the

bonding performance of these adhesive systems. The results indicated that the two etch-and-rinse self-priming adhesives exhibited thicker hybrid layers than those found in self-etching adhesive systems. The all-in-one adhesive showed droplet formation between the adhesive and the resin composite. The resin tags formed with the etch-and-rinse adhesives were much longer than those found with the self-etching adhesives.

Chang and shin (2010)[13] evaluated the influence of chlorhexidine with different application methods (before and after etching) on the microtensile bond strength to dentin in Class I cavities. The results of the study indicated that chlorhexidine pretreatment did not affect the bond strength of specimens tested at the immediate testing period, regardless of the application method used. However, after 10,000 thermocycles, a significant bond strength reduction was found in the control group. It was concluded that a 2% chlorhexidine application after etching with 37% phosphoric acid produced superior bond strength.

Moon et al (2010)[33] reviewed on the relationship between hybrid layer bond degradation and the Matrix metalloproteinases. These matrix metalloproteinase are released by the dentin when treated with etch and rinse adhesives and self etching adhesives, which can reduce the bond stability over time. MMP-2, MMP-8 and MMP-9 are indicated as the active proteases that breakdown the collagen fibrils in the hybrid bond layer. Dentin bonding procedures utilizing Chlorhexidine for different application times and

concentrations have been developed, as chlorhexidine is a known MMP inhibitor. The application of 2% Chlorhexidine to the phosphoric acid etched surface after rinsing off the acid is the only procedure that has been successful when clinically tested for longer periods of time. This pre treatment has shown to prevent resin dentin bond degradation. It was concluded that the adoption of pre-treatment of the dentin with chlorhexidine has been recommended as means of improving bond stability.

Mjor et al (2011)[32] analysed the reasons for replacement of restorations in permanent teeth in general dental practice. Variations in parameters were noted in the selection of restorative material and also in the longevity of restoration. A survey of 24,429 restorations placed by 243 Norwegian dentists in general practice was assessed. Results indicated that the reasons for replacement of restorations in permanent teeth in patients were secondary caries, fracture of restoration due to adhesive failure in composites and discolouration in composite, amalgam and glass ionomer restorations over a period of time.

Stanislawczuk et al (2011)[44] evaluated the effect of 2% Chlorhexidine digluconate on immediate and 2-year resin–dentin bond strength and the silver nitrate uptake of two etch-and-rinse adhesives when applied in aqueous or associated to the phosphoric acid conditioner. Based on the results of the current study it was concluded that 2% CHX application, either in an aqueous solution

or associated with the acid conditioner, preserves durability of the resin–dentin interface even after 2 years of water storage.

Van meerbeek et al (2011)[54] reviewed on the latest developments with regard to the self-etch approach. The author described the general characteristics of self-etch adhesives and the major shortcomings of the most simple-to-use one-step (self-etch) adhesives. The author described the actual bonding performance of the self etch adhesives. It depends on the composition of the adhesive, mostly the presence of the specific functional monomer. The fundamental mechanism of the bonding occurring in these self etch adhesive systems are described based on the AD concept. It was concluded that phosphoric-acid etching of dentin could nowadays be considered too aggressive for dentin, given all the consequences related to exposure of the vulnerable collagen. Hence the use of mild self etch adhesives are deemed better. Mild self-etch adhesive used should contain functional monomers with a high chemical affinity to hydroxyapatite to exhibit superior bonding efficacy.

Lafuente D (2012)[27] evaluated the appearance of the hybrid layer of teeth bonded with one total and one self etch bonding, treated with and without 2% chlorhexidine after aging for four months in water. The results indicated that all the groups treated with chlorhexidine had the presence of a uniform hybrid layer, than the groups without chlorhexidine. It was concluded that the use of 2% chlorhexidine reduced the deterioration of hybrid layer when stored in water.

MATERIALS AND METHODS

ARMAMENTARIUM (Figure 1)

- Diamond saw
- #600-grit silicon-carbide paper
- Tweezers
- Absorbant paper
- Microbrush
- Plastic instrument
- Custom made aluminium jig
- Anodized aluminium composite instrument (Premier dental products, USA)
- Micromotor hand piece (NSK, Contra angled hand piece latch type, Japan)
- Polywave - LED Curing lamp- (Bluephase, Ivoclar Vivadent AG, Liechtenstein, Germany)
- Hard tissue microtome (Leica, Germany)
- Universal testing machine (Instron, Norwood, USA)
- Scanning Electron Microscope (FEI, Quanta 250, USA)

MATERIALS USED (Figure 2)

- G-bond (GC, Tokyo, Japan)
- Clearfil S3 Bond (Kuraray, Tokyo, Japan)
- 2% Chlorhexidine Digluconate (Asep RC, Anabond Stedman, India)
- Filtek (Z250 XT, 3M ESPE, St Paul, MN, USA)
- Saline (0.9% w/v sodium chloride injection, NS, Baxter, India)

Figure 1: Armamentarium

Figure 2: Materials used

TEETH SELECTION:

Forty extracted, non- carious human maxillary premolars were used for the current study. Teeth indicated for orthodontic extraction were selected for the study, and were obtained with informed consent from the patients. The teeth thus obtained were then disinfected in 0.5% chloramine solution, stored in distilled water and used within 6 months after extraction. The occlusal enamel of the teeth was removed perpendicular to the long axis of each tooth using a low-speed diamond saw under water cooling. The enamel-free, exposed dentin surfaces were further polished with wet #600-grit silicon-carbide paper for 60 s to standardize the smear layer.

EXPERIMENTAL DESIGN:

Two ultra mild self etch (single step) adhesive systems were used for the current study. One of the adhesive systems used was HEMA-free (**G-bond**, **GC, Tokyo, Japan**) containing two principal functional monomers, being the '4-MET' monomer (4-metacryloxyethyl trimellitic acid) and the 'Pam' monomer, representing a proprietary phosphoric acid monomer. The other adhesive system used was HEMA-rich (**Clearfil S3 Bond**, **Kuraray, Tokyo, Japan**) containing a 10 MDP functional monomer. Chlorhexidine selected for the current study was 2% Chlorhexidine digluconate (**Asep RC, Anabond Stedman, India**) (*Figure 2*)

The prepared teeth were then randomly divided into 4 equal groups comprising of 10 teeth in each group

Group I: Clearfil S3 Bond

Group II: Clearfil S3 Bond with 2% Chlorhexidine Digluconate Solution

Group III: G-Bond

Group IV: G-Bond with 2% Chlorhexidine Digluconate Solution

BONDING PROCEDURES:

The adhesive systems used were applied onto the prepared dentin surfaces following the respective manufacturers' instructions.

GROUP I: Clearfil S3 Bond

Clearfil S3 Bond (Kuraray, Tokyo, Japan), adhesive was applied using a microbrush onto the prepared tooth surface and left undisturbed for about 20 seconds. The entire adherent surface was then dried using a steady stream of high pressure air (30 psi) for 5 seconds to obtain a thin evenly spread layer of adhesive over the prepared dentin surface.

GROUP II: Clearfil S3 Bond + 2% CHX Digluconate

The adhesive was applied onto the tooth surface as in group I; however a **2% Chlorhexidine Digluconate (Asep RC, Anabond Stedman, India)** was applied prior to the application of the adhesive. The chlorhexidine solution was flushed to the dentin using a syringe for 60 seconds, left undisturbed for 60 seconds without being rinsed, and then dried with absorbent paper. This application time and CHX concentration was adopted because no study had addressed the effectiveness of lower CHX concentration and application times [6, 29].

GROUP III: G Bond

The adhesive **G Bond (GC, Tokyo, Japan)** was applied using a microbrush onto the prepared tooth surface and left undisturbed for 5-10 seconds followed by drying the prepared tooth surface thoroughly under

maximum air pressure (30 psi) for 5 seconds leaving the adhesive layer that is substantially more hydrophobic.

GROUP IV: G Bond + 2% CHX Digluconate

The prepared tooth surface was conditioned with the use of **2% Chlorhexidine Digluconate (Asep RC, Anabond Stedman, India)** preceding the application of the adhesive. The chlorhexidine solution was applied to the dentin, rested for 60 seconds without being rinsed, and dried with absorbent paper. Then the adhesive was applied onto the tooth as in group III.

The adhesives were then light-activated with a polywave LED curing light at 1200 mW/cm2 for 10 s (**Bluephase, Ivoclar Vivadent AG, Liechtenstein, Germany**). Resin composite build-ups (**Filtek Z250 XT, 3M ESPE, St Paul, MN, USA**) were placed on the bonded surfaces (3 increments of 1.5 mm each) that were individually light activated for 20 s each. All the bonding procedures were carried out by a single operator in a room with controlled temperature and humidity. The teeth were then placed in distilled water at 37°C for a week.

PREPARATION OF SPECIMEN FOR MICROTENSILE TESTING:

The roots of the teeth were then sectioned off and coronal portion were mounted in acrylic resin (*Figure 4*). This acrylic block was mounted on a hard tissue microtome (*Figure 3*) to be sectioned. The teeth were longitudinally sectioned in both ‘‘x’’ and ‘‘y’’ directions across the bonded interface under

water cooling to obtain bonded sticks with a cross sectional area of approximately 1.0 ± 0.1 mm^2 (*Figure 5*). Resin–dentin sticks were obtained in accordance with the non-trimming technique [42]. Using this non-trimming technique first described by Shono et al [42], the beams were prepared, with resin composite comprising the upper half of the beam and dentin comprising the lower half. Four resin dentin sticks were acquired from each tooth sample by this method, thereby making a total of 40 dentin composite specimens in each group.

Figure 3: Hard tissue microtome

Figure 4: Tooth sections mounted in acrylic blocks

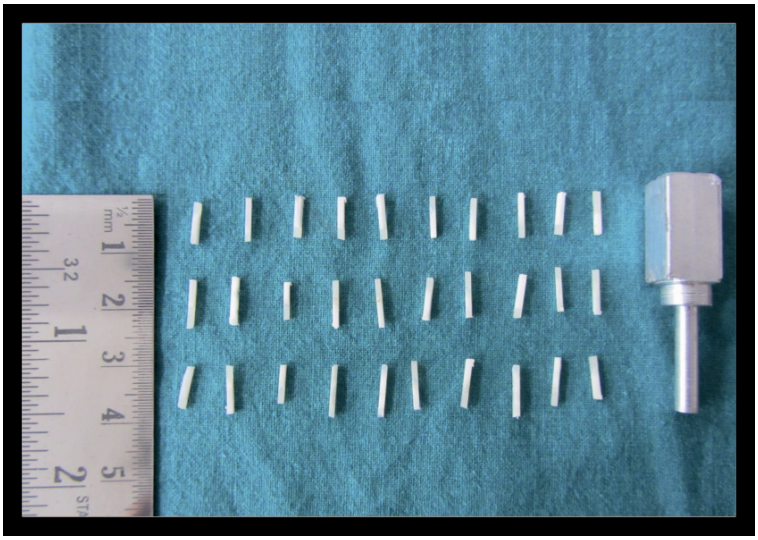


Figure 5: Resin dentin sticks of size 1.0 ± 0.1 mm^2 after sectioning

MICROTENSILE BOND STRENGTH TESTING:

Each bonded stick was then attached to a custom made jig with cyanoacrylate resin (*Figure 6*) for microtensile bond strength testing and subjected to a tensile force in a Universal testing machine (*Figure 8*) (Instron, Norwood, USA) (*Figure7*) at a cross head speed of 1 mm/min. The load at which the failure occurred was recorded by specialized software attached to the universal testing machine. Representative samples from each group were selected to be observed under the scanning electron microscope (SEM). (*Figure 9*)

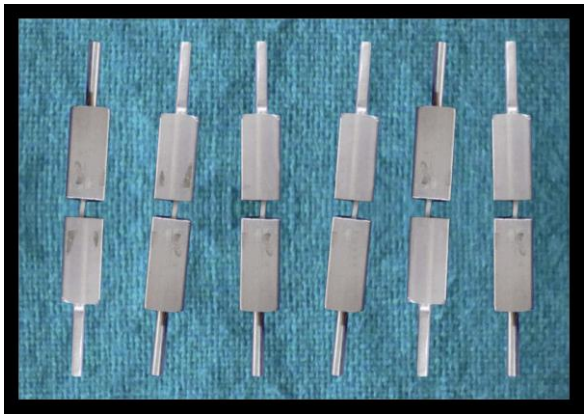


Figure 6: Resin dentin sticks mounted on jig

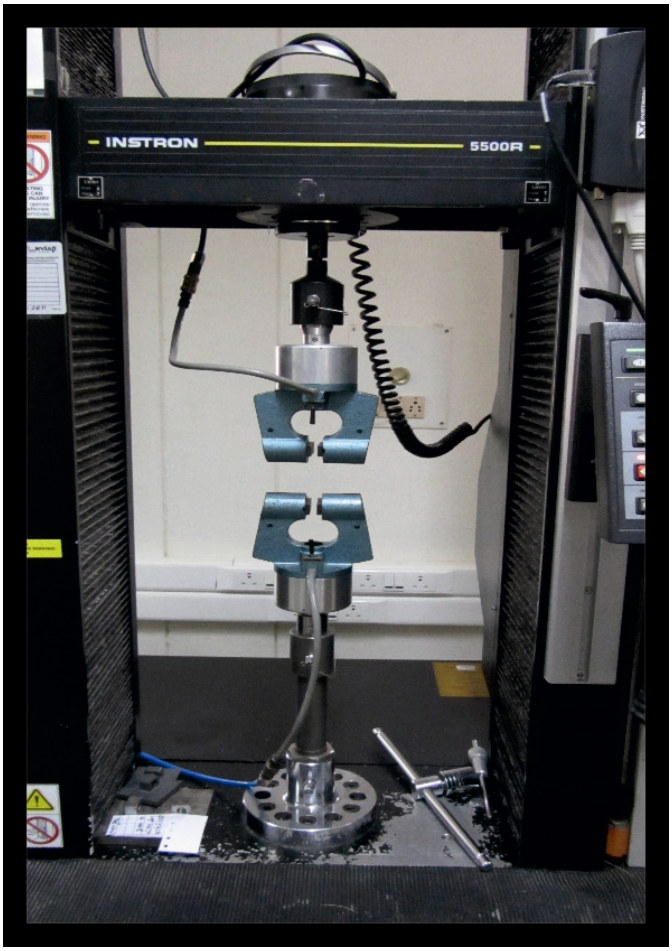


Figure 7: Universal testing machine (Instron, Norwood, USA)

Figure 8: Specimen mounted on universal testing machine

SCANNING ELECTRON MICROSCOPY:

All fractured specimens were dried at room temperature for 24 hrs in a dessicator and sputter coated with gold (*Figure 10*). Both surfaces of each fracture site were observed under a scanning electron microscope with 2000x-4000x magnification at 15 kV (FEI, Quanta 250, USA). The fracture modes were classified as described by Hashimoto et al (2000) viz (1) cohesive failure in the composite, (2) cohesive failure in the adhesive resin, (3) adhesive failure (4) mixed failures.

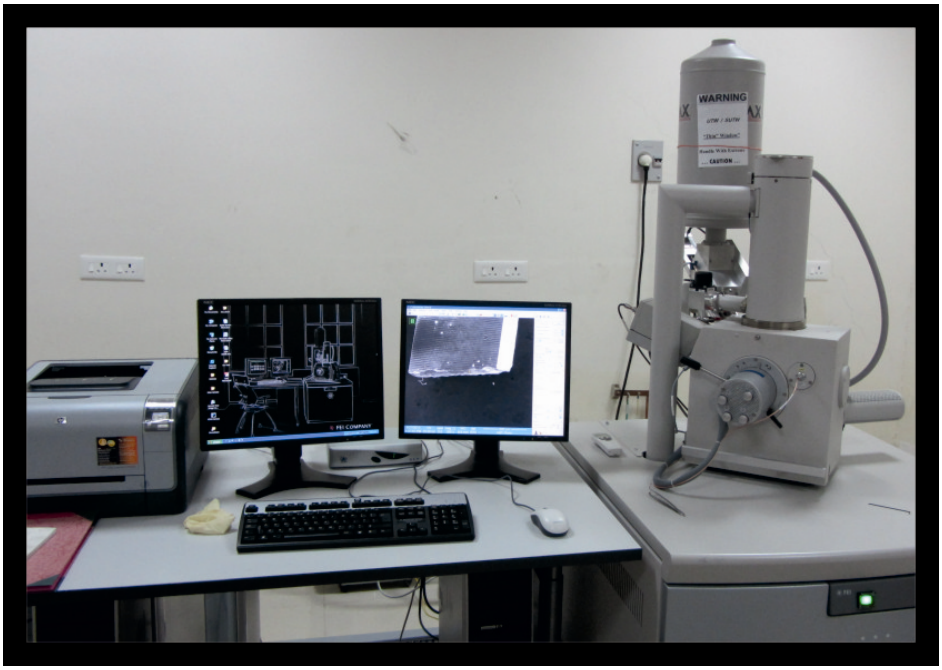


Figure 9: Scanning electron microscope (FEI, Quanta 250, USA)

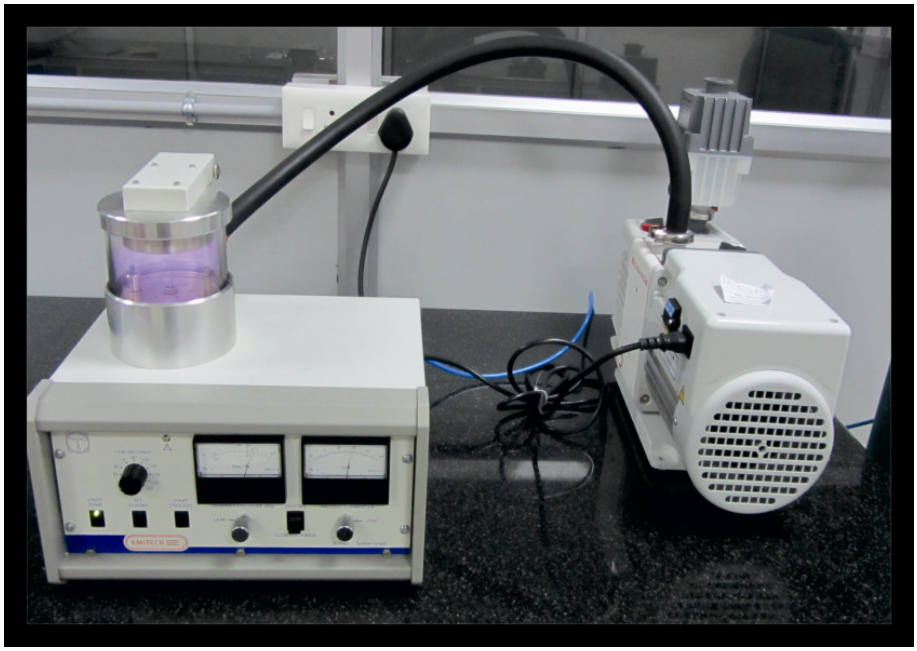


Figure 10: Specimen placed for gold sputter coating

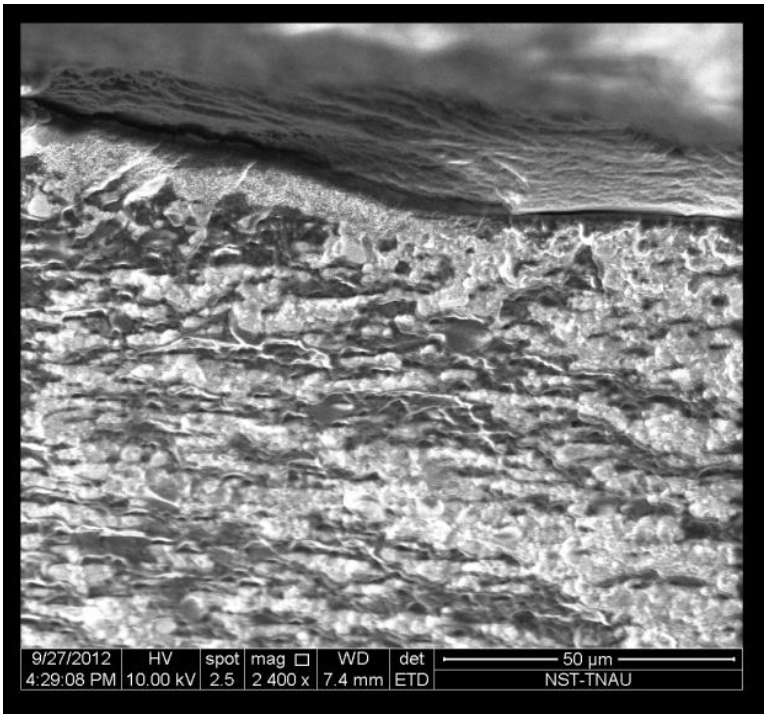


Figure 11: SEM micrograph showing hybrid layer in Clearfil S3 bond with CHX

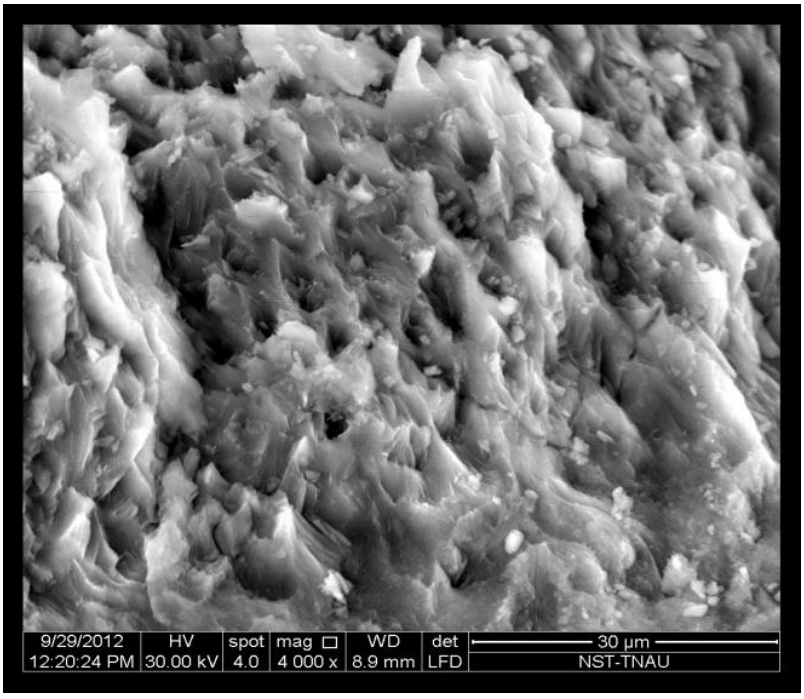


Figure 12: SEM micrograph showing resin tags in Clearfil S3 bond with CHX

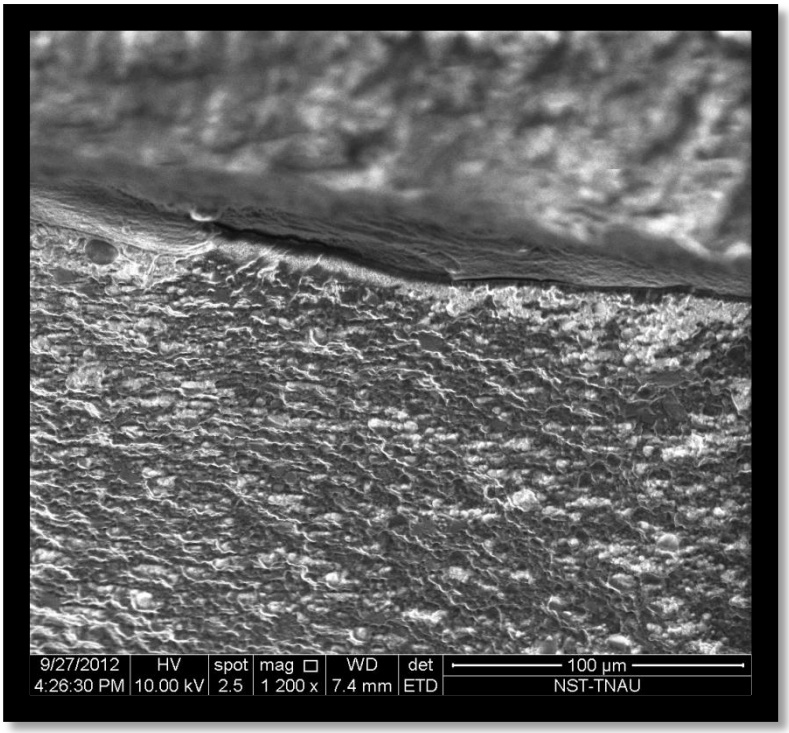


Figure 13: SEM micrograph showing hybrid layer in Clearfil s3 bond without CHX

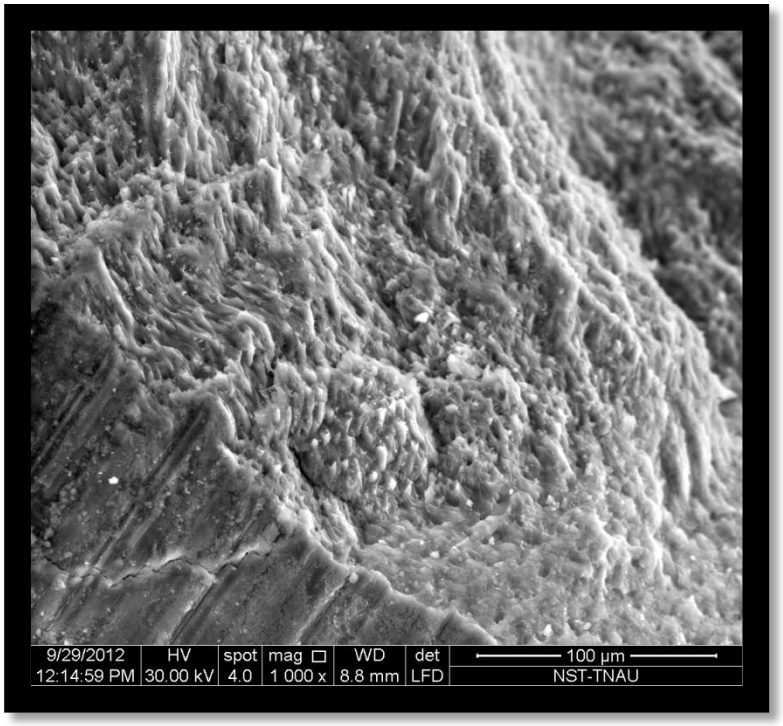


Figure 14: SEM micrograph showing resin tags in Clearfil s3 bond without CHX

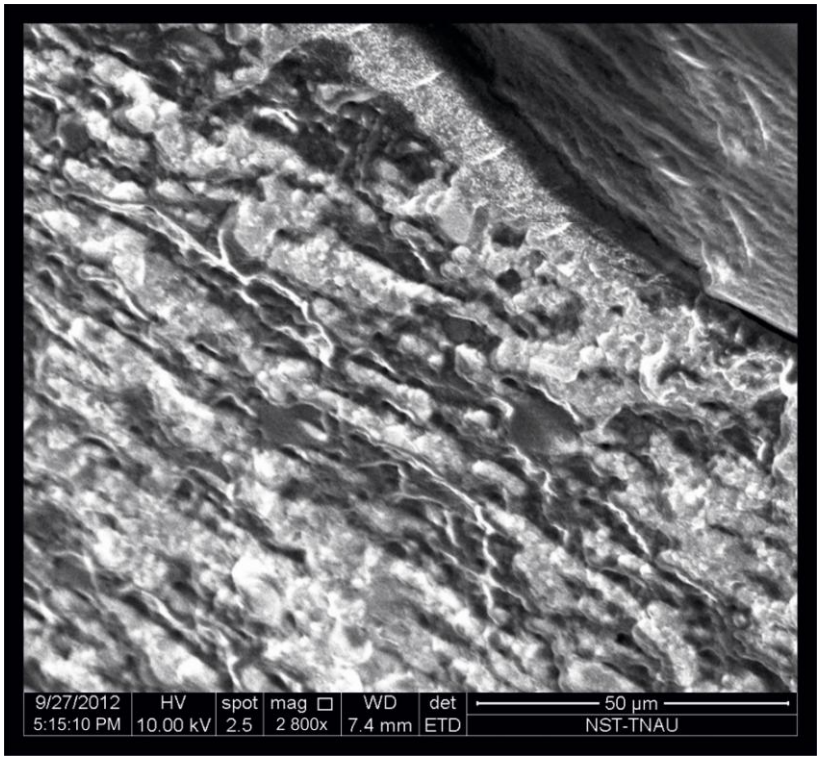


Figure 15: SEM Micrograph showing Hybrid layer in G bond with CHX

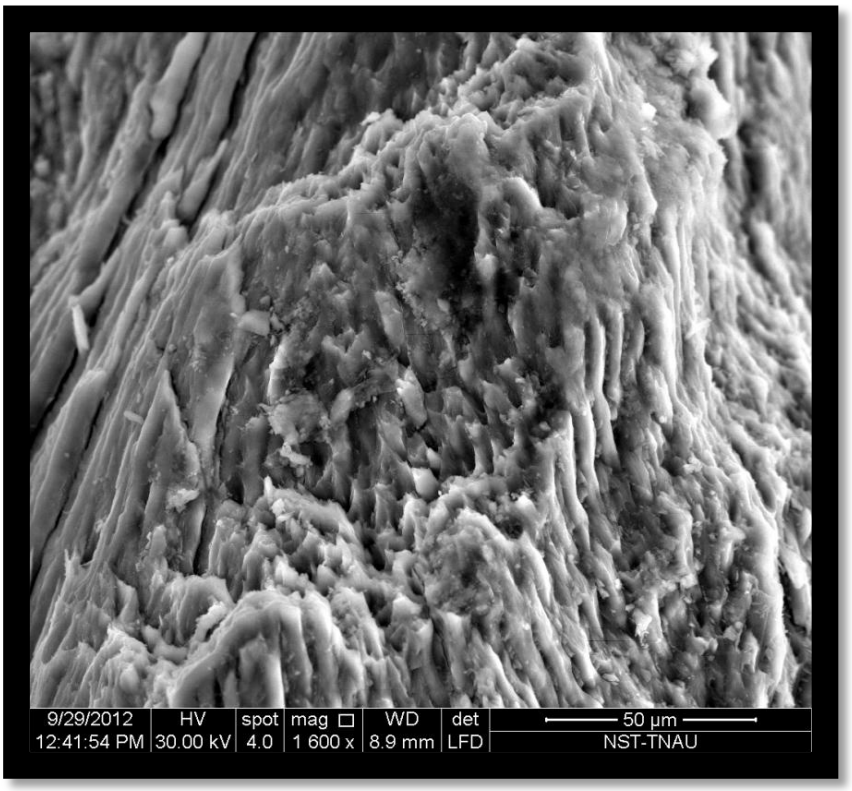


Figure 16: SEM Micrograph showing resin tags in G Bond with CHX

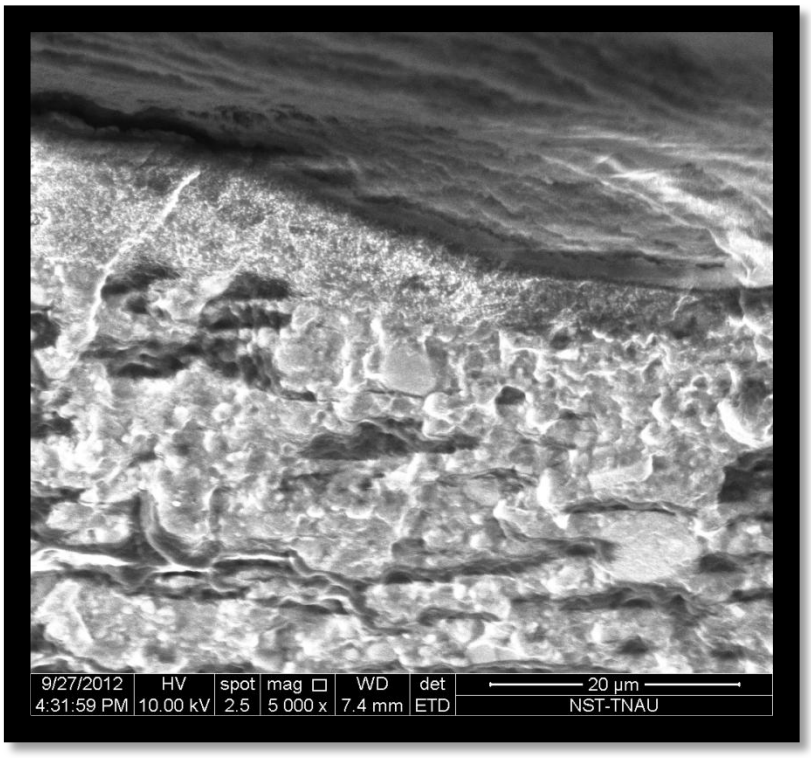


Figure 17: SEM Micrograph showing hybrid layer in G bond without CHX

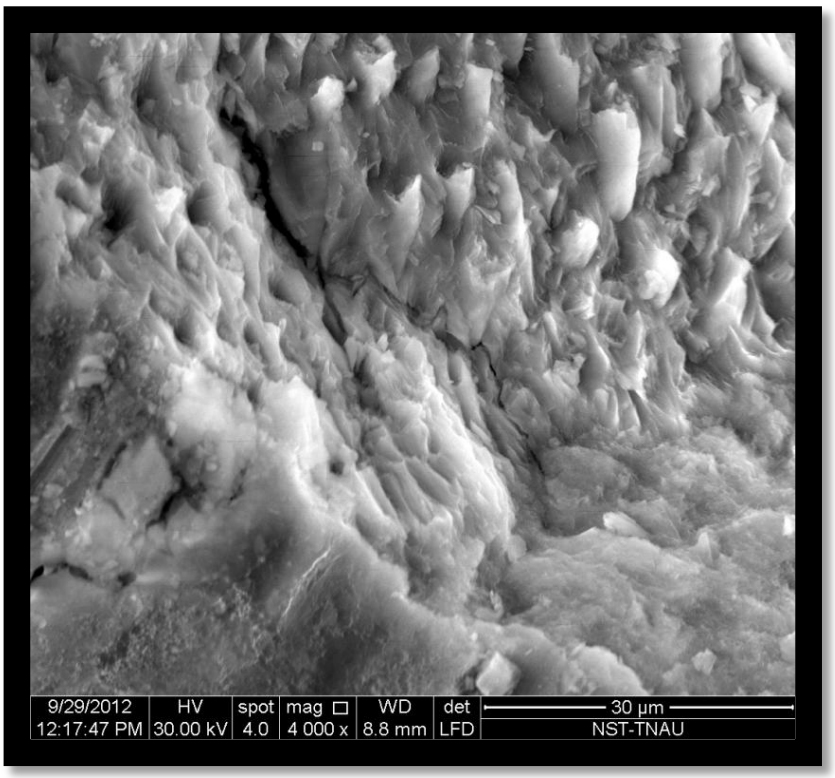


Figure 18: SEM Micrograph showing resin tags in G Bond without CHX

STATISTICAL ANALYSIS

The statistical analysis for the values recorded after microtensile bond strength testing was performed with the software ***SPSS version 17.0***. The values tabulated were tested for significance using student's t test with the level of significance set at $p < 0.05$.

RESULTS

MASTER CHART

Microtensile bond strength values in (Mpa)

Sl No:	Groups			
	I	II	III	IV
1	12.57	4.89	3.37	6.46
2	10.48	6.48	4.92	5.12
3	13.82	4.21	5.24	6.54
4	13.43	8.63	6.85	5.08
5	12.82	8.42	4.96	4.98
6	12.89	6.87	4.87	4.02
7	13.48	6.90	3.98	4.47
8	12.62	5.90	3.99	6.78
9	12.87	6.91	4.90	5.83
10	13.92	3.89	6.12	5.42
Mean value	12.89	6.31	4.92	5.47

Group I – Clearfil S3 Bond

Group II – Clearfil S3 Bond with 2% Chlorhexidine Digluconate

Group III – G Bond

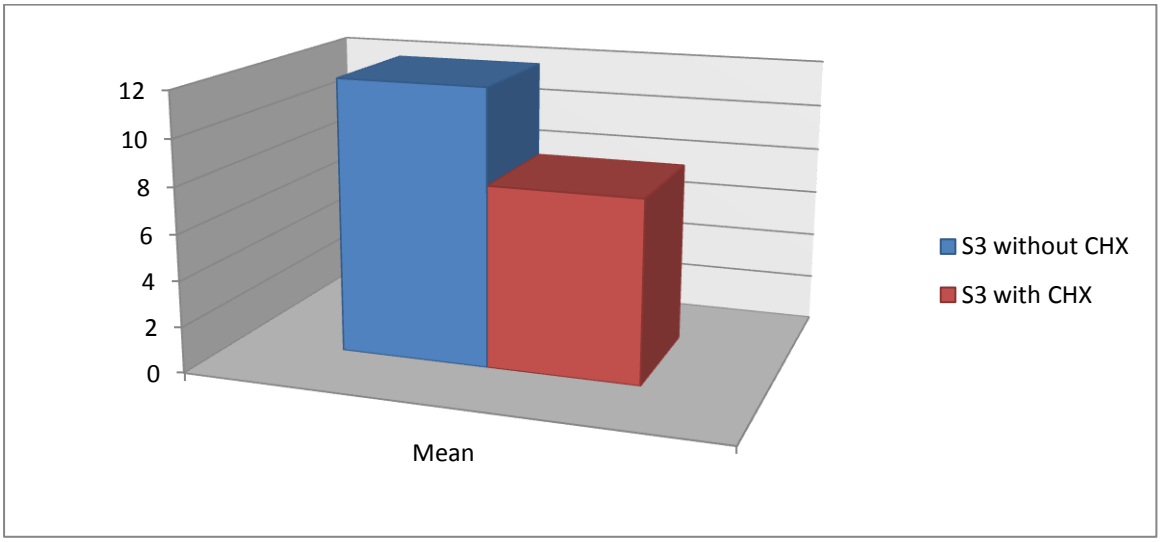
Group IV– G Bond with 2% Chlorhexidine Digluconate

Table I: Mean difference in bond strength values between S3 Bond with and without Chlorhexidine

ADHESIVES	N	MEAN	STD. DEVIATION	t-VALUE	LEVEL OF SIGNIFICANCE
Maximum load					
S3 Bond without CHX	15	12.8982	8.54421	2.187	0.037*
S3 Bond with CHX	15	6.3173	7.92181		

(*- significant)

Graph I: Bar diagram representing Mean values between Clearfil S3 bond with and without CHX



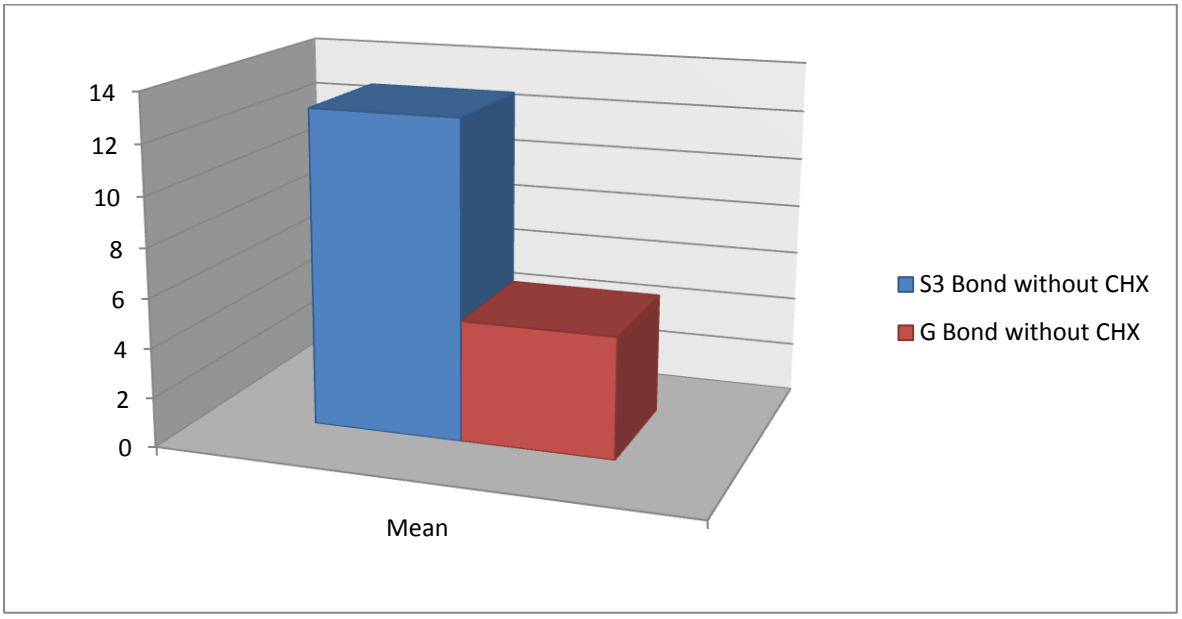
In the above table I the 't' value (2.187) for the mean difference in the microtensile bond strength values between S3 Bond without chlorhexidine and S3 Bond with Chlorhexidine is significant ($p<0.037$). The mean microtensile bond strength values of S3 bond without Chlorhexidine and S3 Bond with Chlorhexidine were 12.8982 and 6.6173 respectively. Hence it can be inferred that S3 bond without Chlorhexidine had better bond strength when compared to S3 bond with Chlorhexidine.

Table II: Mean difference in bond strength values between S3 Bond and G Bond without Chlorhexidine

ADHESIVES	N	MEAN	STD. DEVIATION	t- VALUE	LEVEL OF SIGNIFICANCE
Maximum load					
S3 Bond without CHX	15	12.8982	8.54421	2.957	0.006**
G Bond without CHX	15	4.9247	6.00648		

(** - Highly significant)

Graph II: Bar diagram representing Mean values between Clearfil S3 bond and G bond without CHX



In the above table II the 't' value (2.957) for the mean difference in the microtensile bond strength values between S3 Bond without Chlorhexidine and G Bond without Chlorhexidine is highly significant ($p<0.006$). The mean microtensile bond strength values of S3 bond without Chlorhexidine and G Bond without

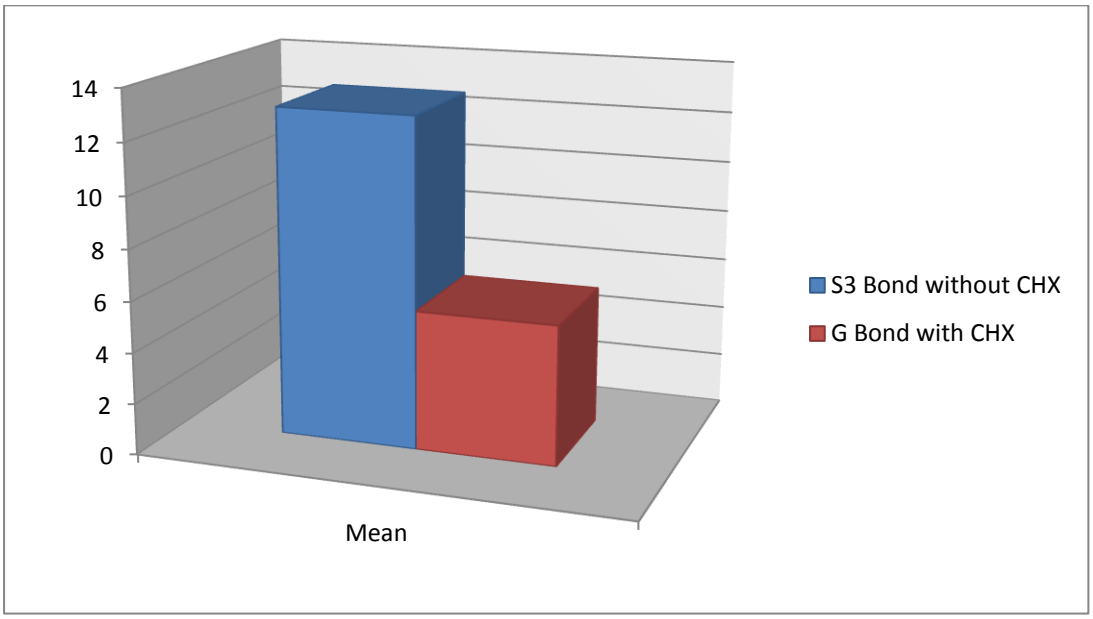
Chlorhexidine were 12.8982 and 4.9247 respectively. Hence it can be concluded that S3 Bond without Chlorhexidine had better bond strength when compared to G Bond without Chlorhexidine.

Table III: Mean Difference in bond strength values between S3 Bond without Chlorhexidine and G Bond with Chlorhexidine

ADHESIVES	N	MEAN	STD. DEVIATION	t- VALUE	LEVEL OF SIGNIFICANCE
Maximum load					
S3 Bond without CHX	15	12.8982	8.54421	2.543	0.017*
G Bond with CHX	15	5.4787	6.00648		

(*- Significant)

Graph III: Bar diagram representing mean values between Clearfil S3 bond without CHX and G Bond with CHX



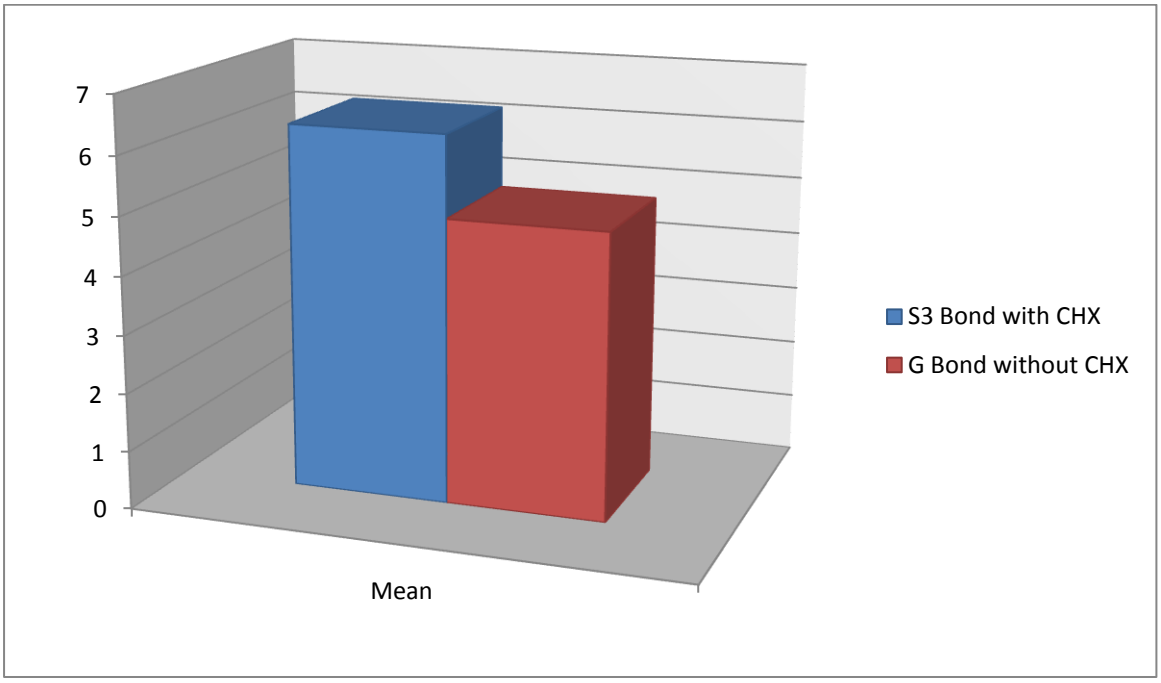
In the above table III the 't' value (2.543) for the mean difference in the microtensile bond strength values between S3 Bond without Chlorhexidine and G Bond with Chlorhexidine is significant ($p<0.017$). The mean microtensile bond strength values of S3 bond without Chlorhexidine and G Bond with Chlorhexidine were 12.8982 and 5.4787 respectively. Hence it can be inferred that S3 Bond without Chlorhexidine had better bond strength when compared to G Bond with Chlorhexidine.

Table IV: Mean difference in bond strength values between S3 Bond with Chlorhexidine and G Bond without Chlorhexidine

ADHESIVES	N	MEAN	STD. DEVIATION	t- VALUE	LEVEL OF SIGNIFICANCE
Maximum load					
S3 Bond with CHX	15	6.3173	7.92181	0.543	0.592 NS
G Bond without CHX	15	4.9247	6.00648		

(NS- Not Significant)

Graph IV: Bar diagram representing mean values between Clearfil S3 bond with CHX and G Bond without CHX



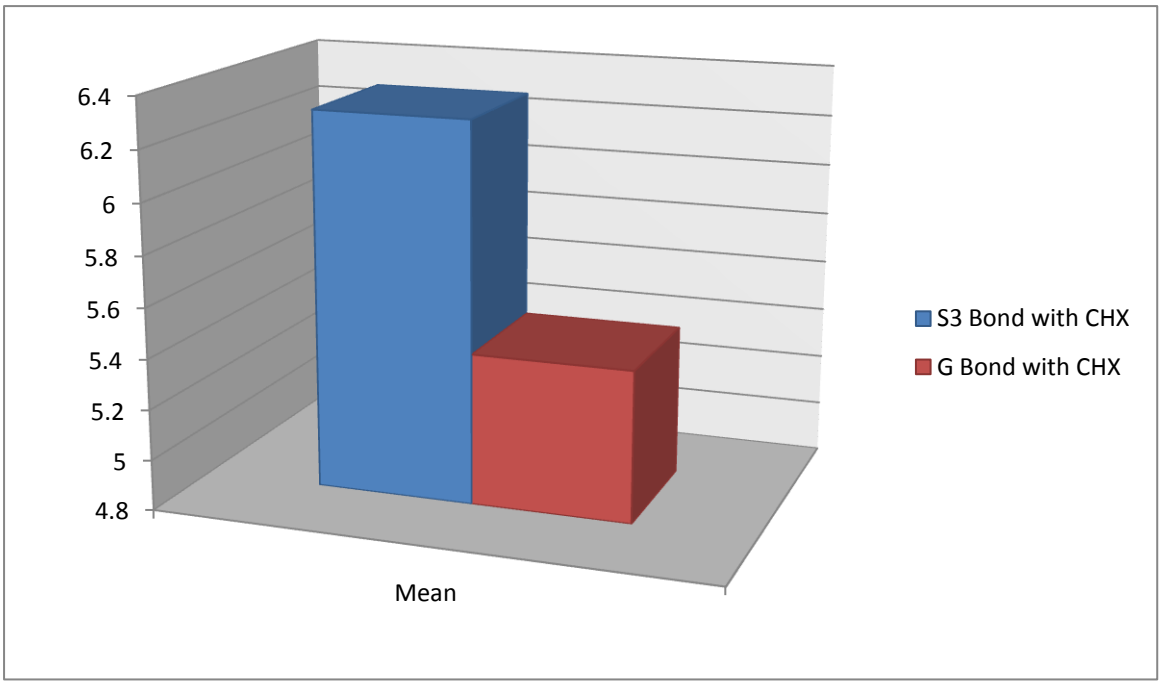
In the above table IV the 't' value (0.543) for the mean difference in the microtensile bond strength values between S3 Bond with chlorhexidine and G Bond without Chlorhexidine is not significant (p= 0.592) . Hence it can be concluded that the bond strength of S3 bond with chlorhexidine and G bond without chlorhexidine are similar.

Table V: Mean difference in bond strength values between S3 Bond with Chlorhexidine and G Bond without Chlorhexidine

ADHESIVES	N	MEAN	STD. DEVIATION	t- VALUE	LEVEL OF SIGNIFICANCE
Maximum load					
S3 Bond with CHX	15	6.3173	7.92181	0.300	0.767 ^{NS}
G Bond with CHX	15	5.4787	7.39549		

(NS- Not significant)

Graph V: Bar diagram representing mean values between Clearfil S3 bond and G Bond with CHX



In the above table V the 't' value (0.300) for the mean difference in the microtensile bond strength values between S3 Bond with Chlorhexidine and G

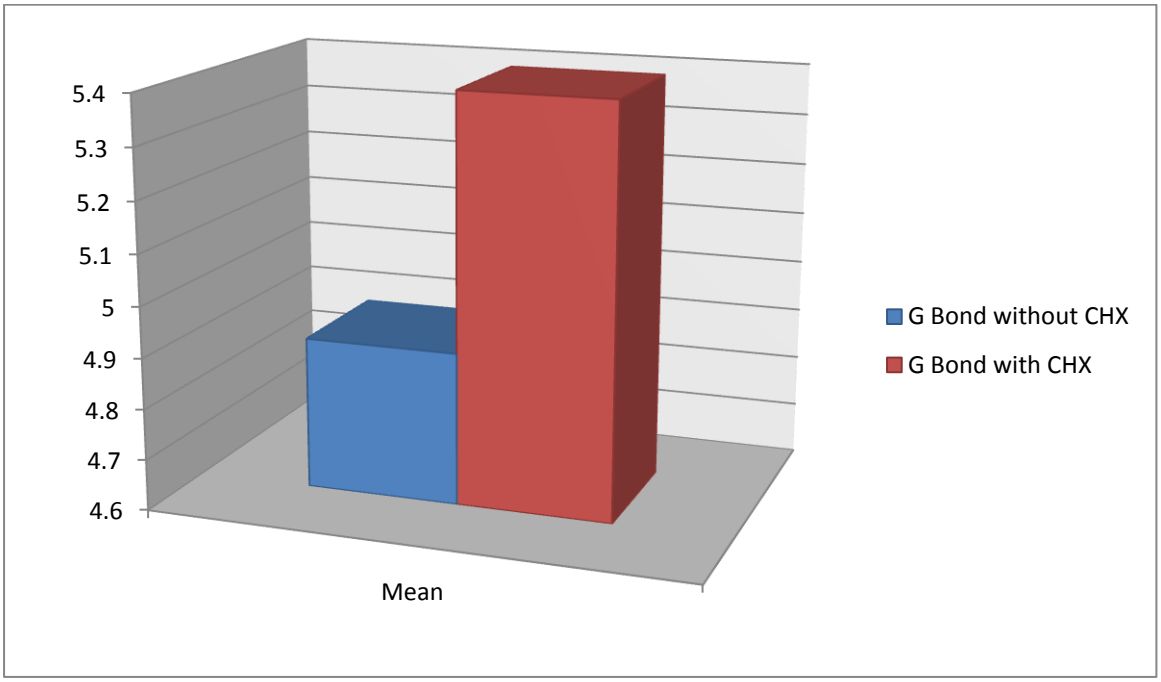
Bond with Chlorhexidine is not significant (p=0.767) . Hence the bond strength between S3 bond and G bond with Chlorhexidine are similar.

Table VI: Mean difference in bond strength values between G Bond with and without Chlorhexidine

ADHESIVES	N	MEAN	STD. DEVIATION	t-VALUE	LEVEL OF SIGNIFICANCE
Maximum load					
G Bond without CHX	15	4.9247	6.00648	0.225	0.823 NS
G Bond with CHX	15	5.4787	7.39549		

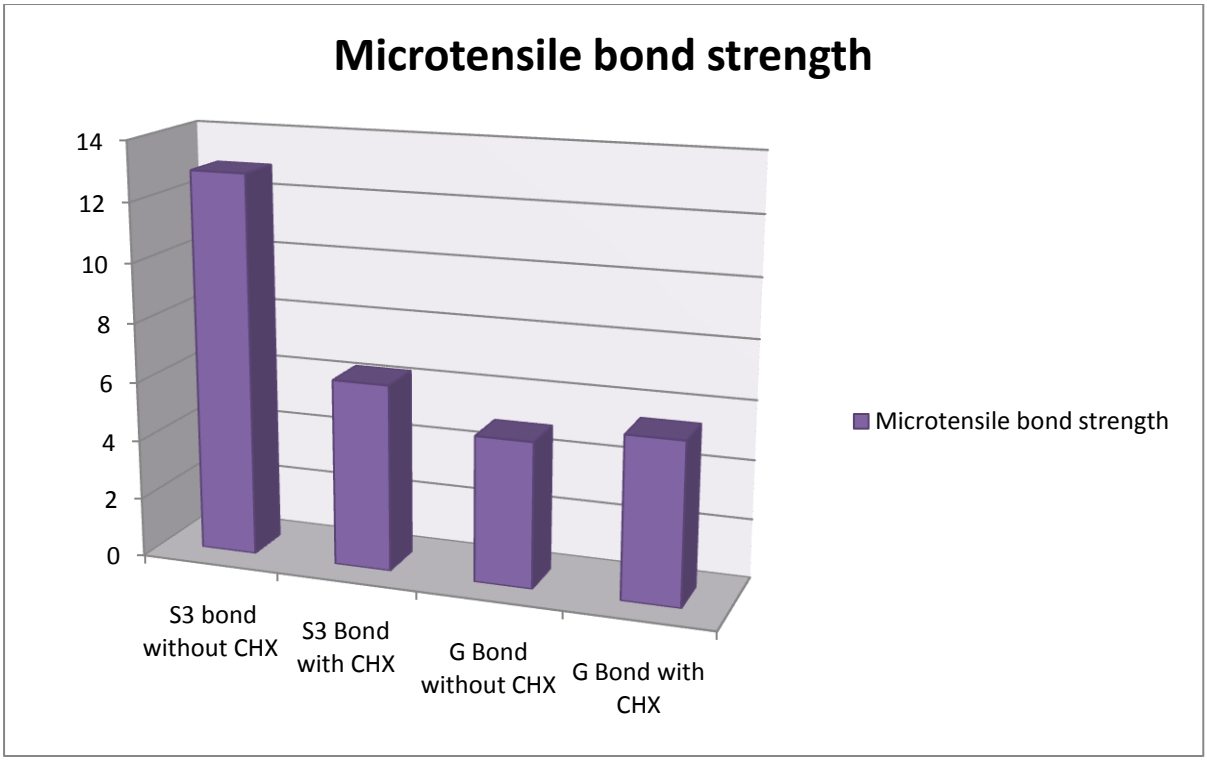
(NS- Not significant)

Graph VI: Bar diagram representing mean values between G bond with and without CHX



In the above table VI the 't' value (0.823) for the mean difference in the microtensile bond strength values between G Bond without Chlorhexidine and G Bond with Chlorhexidine is not significant (p=0.823). Hence indicating that the bond strength between the G Bond with and without chlorhexidine are alike.

Graphical representation of microtensile bond strength of the 4 groups



DISCUSSION

Adhesive technology has evolved rapidly since its inception more than fifty years ago. The modern restorative techniques are based on the adhesive properties of tooth coloured resin-based materials. Following the pioneer approach of Buonocore in 1955 [7], researchers and manufacturers have enhanced both sealing and bonding capabilities of dental adhesives. The foremost challenge for dental adhesives is to provide an equally effective bond to two hard tissues of different nature. Bonding to enamel has been established to be durable, but bonding to dentin is far more intricate and can apparently only be achieved when more complicated and time-consuming application procedures are followed. Despite significant improvements of adhesive systems, the bonded interface still remains the weakest area of tooth-coloured restorations.

Present day adhesives are often regarded as technique-sensitive with the smallest error in the clinical application procedure being penalized by marginal discolorations, poor marginal adaptation and subsequent loss of retention of the restoration [30, 31]. This led to the demand for simpler, more user-friendly and less technique- sensitive adhesives. As a consequence, the manufacturers are driven into developing newer adhesives at a rapid pace. Currently marketed dental adhesives can be divided into two categories based on the interaction with enamel/dentin: (1) etch-and-rinse adhesive systems that remove the smear layer [47,51] (2) self etch technology consisting of self-etching primer with an acidic monomer that maintains the smear layer as substrate for bonding [47, 51]. The self-

etching systems that are marketed can further be classified as two-step and one-step types (which are available in all-in-one or one-bottle systems), according to the resin application time to the tooth surface.

The self etching adhesives are alleged to be user-friendly (shorter application time with fewer steps) and less technique-sensitive (no wet-bonding, simple drying), thereby resulting in a reliable clinical performance, though this appeared very product-dependent. Another important clinical benefit of self-etch adhesives is the absence of or at least lower incidence of post-operative sensitivity experienced by patients as compared to that associated with etch-and-rinse adhesives. This should to a great extent be attributed to their less aggressive, with respect to dentin and as that compared to phosphoric-acid etching and thus more superficial interaction with dentin. Hence leaving the tubules largely obstructed with smear. All these favourable key-features have lead to the progressively growing popularity of self-etch adhesives in today's dental practice. Therefore self etch adhesives were selected for the current study.

Bonding by the adhesive systems is formed by the impregnation of blends of resin monomers into the dentin substrate. The stability of the bonded interface relies on the creation of a compact and a more homogenous hybrid layer. In the etch-and-rinse strategy after the preliminary etching to demineralize the substrate, bonding monomers impregnate the porous etched substrate [34, 52]. The self-etch approach uses acidic adhesive co-monomers that

simultaneously demineralize and infiltrate dentin; theoretically ensuring complete penetration of the adhesive [12]. Adhesive stability is related to the effective coupling of the co-monomers present in the adhesive with the infiltrated substrate. Previous studies have evaluated the thickness of the hybrid layer between total etch and etch and rinse adhesives. It has been proved that the hybrid layers that are created by the etch-and-rinse adhesives were thicker than those observed in the specimens that are bonded with self-etching adhesive systems[1]. Despite the thinner hybridized complex, higher immediate bond strength has been reported for these self-etching systems [36, 50.] This suggests that the presence of uniform demineralization front which is completely impregnated by resin is the factor necessary for bonding efficacy rather than the thickness of the hybrid layer [46].

The morphological feature of the adhesive tooth interface produced by self-etch adhesives depends to a greater extent on the manner their functional monomers interact with the dental substrate [16]. The actual interaction depth of self-etch adhesives at dentin depends on the pH of the self etch solutions. It differs from a few hundreds of nanometers in case of an 'ultra-mild' self etch approach (pH > 2.5), which is referred to as 'nano-interaction' [26]. The interaction depth is about 1μm for a 'mild' self-etch approach (pH≈2), a depth between 1 and 2μm for a 'strong' self-etch approach (pH between 1 and 2), and to an interaction of several micrometers deep for a 'strong' self-etch approach

(pH≤1). Each manufacturer uses different functional monomers for their adhesives being marketed.

The bonding mechanism of the ultra mild self etch adhesives is also based on the mechanism of hybridization as in etch and rinse adhesives. The difference in ultra mild self etch adhesives is that they produce a hybrid layer of just a few nanometers such that the collagen fibrils are not completely deprived from the hydroxyapatite which might serve as a receptor for additional interaction of these functional monomers. Thereby it was hypothesized that this may lead to enhanced bond strength of these adhesives. Hence ultra mild self etch adhesives were selected for the current study.

The actual bonding performance acquired by self-etch adhesives varies to a great extent. This difference depends not only on the actual class of self-etch adhesives but certainly also on the actual composition. They are more specifically based on the actual functional monomer included in the adhesive formulation. Specific functional monomers, such as 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), 4-MET (or 4-methacryloxyethyl trimellitic acid) and phenyl-P (2-methacryloyloxyethyl phenyl hydrogen phosphate) are in use in these self etch adhesives. Clearfil S3 Bond manufactured by Kuraray dental uses 10 MDP as the functional monomer in its composition. Likewise G bond manufactured by GC Tokyo uses 4 MET with Phenyl P as its functional monomer. Therefore these two ultra mild self etch

adhesives with different functional monomers (10 MDP and 4 MET with phenyl P) were selected for the current study.

Despite the adhesive approach itself, the result of loss in bond strength due to failures within the hybrid layer is often attributed to incomplete hybridization of the dentin surface. This incomplete hybridization leaves the collagen fibrils unprotected and vulnerable to hydrolytic degradation [45] that also is susceptible to other degradation promoting factors such as residual solvent of the adhesive [58, 35] or insufficiently removed surface water, significantly affecting the longevity of the restoration [15, 5]. Recent studies have revealed the contribution of host-derived proteinases (Matrix metalloproteins) to the breakdown of the collagen matrices in the pathogenesis of dentin caries [49] and periodontal disease, with potential and relevant implications in dentin bonding [38]. Ferrari and Tay [18] demonstrated that nanoleakage can occur in the absence of gaps along the resin–dentin interfaces (in vivo). This suggests that the degradation of incompletely infiltrated zones by host-derived proteinases within the dentin matrix may proceed in the absence of bacterial enzymes.[48] Pashley et al [38] reported that acid-etched dentin matrices can be slowly degraded over time by dentin-derived proteolytic enzymes, in the absence of bacteria.

MMPs are a class of zinc- and calcium-dependent endopeptidase [5] trapped within the mineralized dentin matrix during tooth development [49, 56]. They are a group of 23 mammalian enzymes that are capable of degrading all extracellular matrix components. During dentin bonding procedures there is a

release and subsequent activation of these endogenous enzymes which are thought to be responsible for the in vitro thinning and disappearance of collagen fibrils from the incompletely infiltrated hybrid layer. Hence it is hypothesized that the use of an MMP inhibitor could negate the effects caused by these Matrix metalloproteinases.

Chlorhexidine digluconate is a well known antibacterial agent with MMP inhibiting properties that has known to inactivate MMP-2, -8 and -9 [5]. Previous in vitro and in vivo studies have proved that application of 2% Chlorhexidine digluconate postpones the degradation of the hybrid layer (to phosphoric acid etched surface) as when compared to the adhesive interface where no Chlorhexidine is applied [17, 10, 44, 27]. It has been speculated that the MMP's are activated in the dentin when it has been exposed to zinc containing dental materials [19, 33, 28]. Chlorhexidine is thought to chelate to zinc on the active site in turn leading to inhibition of MMPs that have been activated by this mechanism.[33] Hebling et al.[22] showed that hybrid layers from chlorhexidine pre-treated teeth exhibited normal structural integrity of the collagen network compared to the progressive disintegration of the fibrillar collagen network detected in the control teeth. Similarly an in vitro study by Carrillo MR et al revealed that microtensile bond strength created with the use of chlorhexidine as additional primer in an etch-and-rinse adhesive was higher than control specimens after 6 months water storage. [11]

Different clinical approaches have been put forth to improve the infiltration of the monomer and thereby to reduce the rate of water sorption and to reduce the collagen degradation [5]. Hence the use of a 2% Chlorhexidine digluconate rinse before the use of the adhesive was advocated to determine if it has any effect on the immediate bond strength. Short term bond strength testing is done as the substrate is subjected to polymerisation shrinkage stress. Most of the studies applied chlorhexidine in etch-and-rinse adhesives and used chlorhexidine as an additional primer, in which chlorhexidine was applied after or prior to the acid etching step [22, 11, 25, 13] . Only a few studies applied chlorhexidine in self-etching adhesives. Therefore 2% Chlorhexidine Digluconate was used for the current study.

The bonding surface of the tooth was prepared to form a flat dentinal surface. This was done to form a flat uniform layer of dentin adhesive interface. The composite material used for the resin block build up over the adhesive was nanofiller based that has good bonding capabilities to the adhesives used, also it is said to possess better strength when compared to that of the conventional composites. Hence Filtek Z250 nanofilled composite was used for both the adhesive agents.

Bond strength is referred to the force per unit area that is required to debond the adhesive/adherent interface. Microtensile bond strength testing was designed to evaluate the bond strength of small sections of dentin adhesive interface. Sano et al in 1994 [41] introduced microtensile bond strength testing to

dentistry to measure the ultimate tensile strength and modulus of elasticity of mineralized and demineralized dentin. According to Pashley et al, [42] the microtensile bond strength test presents several advantages in comparison to the macro and shear testing that is, it permits a greater number of adhesive failures and the measurement of regional bond strengths. Of all the in vitro tests the microtensile bond strength testing is deemed better, as the bonded interface of smaller cross sectional area of specimens has a better stress distribution during loading. This method has shown to have fewer cohesive failures in dentin or composite than with conventional testing. The major drawback of this test is maintaining the alignment during bonding and testing to avoid stress concentration due to incorrect interfacial geometry. Though this testing comes with its technical limitations, it is still considered to be the most predictable type of in vitro bond strength testing methodology [42, 43, 56]. Considering these factors microtensile bond strength testing methodology was selected for the current study.

The initial specimen design for the microtensile bond strength testing that was proposed by Sano et al [41] was hourglass shaped. Subsequently with increasing popularity of this technique numerous specimen designs were introduced by several researchers [23, 41, 39] of which the stick (square) shaped specimen is the most frequently used. Stick (square) shaped specimens are simpler to prepare when compared to that of the hourglass or dumbbell shaped specimen, and also this design produces a more favorable stress distribution [20].

The advantage of using this non trimming technique is that it allows the study of materials that produce relatively low bond strengths and also more number of samples can be obtained from the same tooth thereby reducing the bias between the samples.

The dimension of the specimen plays a role in the determination of the bond strength. The specimen's dimensions were maintained constant at approximately 1.0 ± 0.1 mm^2 rectangular cross sectional area. Phrukkanon et al.[39] investigated the effect of specimen size and geometry and concluded that the specimen dimensions of about 1.1mm^2 are ideal. Therefore for the current study specimens were prepared to stick (square) shaped with the dimensions of 1.0 ± 0.1 mm^2.

On microtensile bond strength testing the values obtained was tabulated and statistical analysis of Student's t-test was performed. In our study the results indicated that Clearfil S3 bond had better bond strength when compared to that of G bond. This could be attribted to the functional monomer in these adhesives [54]. In case of 4 MET and Phenyl P monomer in the G bond, there is an ionic bond formation of the carboxylic/ phosphate groups of these functional monomers to Ca of hydroxyapatite crystals which was first proved by Yoshida et al [59] in 2004 using X-ray photo-electron spectroscopy (XPS). This chemical bonding potential is insufficient and less stable in the aqueous environment. The functional monomer 10-MDP bonds through its phosphate groups to Hydroxyapatite crystals and forms a regularly layered structure at the surface [60,

[61.] Formation of a 4 nm layered structure called nanolayering was detected which was absent in case of functional monomers 4-MET and phenyl P. In this sense, the chemical bonding promoted by 10-MDP is not only more effective, but also more stable in water than that provided by 4-MET and phenyl-P.

In our study, Clearfil S3 bond without chlorhexidine had significantly better bond strength when compared to Clearfil S3 bond with chlorhexidine. Previous studies have demonstrated that CHX applied had no adverse effects on adhesive bonds to dentin in short term [43, 4]. According to the results of the current study application of 2% CHX resulted in decreased bond strength. It can be speculated that there are interactions among CHX and the adhesive components that may decrease their wettability and the level of dentin conditioning. Also that interaction between the composition of adhesive agent and the CHX may lead to decreased bonding to the dentin substrate leading to reduced bond strength.

The bond strength in case of G bond with and without chlorhexidine was similar suggesting that the addition of chlorhexidine did not affect the immediate bond strength. Many authors have found that the use of chlorhexidine did not produce a negative effect on the bond strength of dentin adhesives when it was used before acid etching as a cavity disinfectant [4, 14]. Chlorhexidine may not be beneficial to all self etching adhesives because the chemistry varies for each adhesive.

Scanning electron microscopic analysis was done in the study to evaluate the mode of failure that occurred in the interface of the debonded samples. The modes of the failure that can occur at the interface are adhesive, cohesive and mixed failures. The SEM observations of the samples showed cohesive failures in the samples with clearfil S3 bond without CHX used as the adhesive whereas in the samples of the tooth with G Bond without CHX, adhesive failures was noted. This signifies that bond failure in the adhesive layer was more prominent in the specimens bonded with G Bond, thereby denoting that the Clearfil S3 bond with a 10 MDP functional monomer has better bonding to the dentin than G Bond with 4 MET and Phenyl P. The SEM observations of the samples of Clearfil S3 bond and G Bond with CHX shows a uniform hybrid layer at the interface. (*Figure 11, 12, 13, 14, 15, 16, 17, 18*)

Within the limitations of the current study, the use of 2% chlorhexidine solution to condition the dentin before the application of the adhesive did not have an adverse effect on the immediate bond strength on teeth bonded with G bond whereas there was reduced bond strength in case of Clearfil S3 bond. However further in vivo studies are to be carried out to clarify, if the use of 2% CHX solution would be able to preserve resin–dentin bonds over longer duration in clinical conditions.

SUMMARY AND CONCLUSION

The current study evaluated the effectiveness of 2% Chlorhexidine digluconate on the immediate microtensile bond strength of two ultra mild self etch adhesives namely Clearfil S3 Bond and G Bond. Four groups of tooth samples were evaluated, **Group I**: Clearfil S3 Bond, **Group II**: Clearfil S3 Bond with 2% Chlorhexidine digluconate Solution, **Group III**: G-Bond, **Group IV**: G-Bond with 2% Chlorhexidine digluconate Solution. After the application of the adhesives on the tooth samples according to their respective groups, composite build up was performed. The samples were then sectioned to obtain resin dentin sticks of 1.0 ± 0.1 mm^2 which were mounted on a jig and tested for microtensile bond strength. The fractured specimens were then viewed under scanning electron microscope and the failure modes were evaluated. The results of the study showed that the use of 2% Chlorhexidine Digluconate before the application of the adhesives did not have an adverse effect on the immediate microtensile bond strength of the specimens bonded with G bond whereas there was a decrease in the bond strength in case of Clearfil S3 bond.

Within the limitations of this study, it can be concluded that the use of 2% Chlorhexidine Digluconate does not have much effect on the immediate microtensile bond strength of the specimens bonded with ultra mild self etch adhesives. However further in vivo studies should be carried out, to assess the long term effects of using 2 % Chlorhexidine digluconate on preservation of resin dentin bonds.

Discovering a method to ensure better and more durable bond strengths between dentin and resinous adhesives can positively affect the durability of an adhesive restoration. This should be the goal we aspire to achieve through various studies and clinical trials such as these.

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